REMARKS

Claims 1 and 3-10 remain before the Examiner for reconsideration.

In the Office Action dated February 11, 2003, The Examiner rejected claims 1 and 5-10 under 35 U.S.C. 102 (b) "as being anticipated by Havens et al for the type of reasons set forth in the previous office actions of 7/16/02 and 10/7/02." Specifically, the Examiner asserted:

> The claims are drawn to a method of increasing loading of active enzyme immobilized in a polyurethane polymer by synthesizing the polymer in a reaction mixture containing water and enzyme to provide an enzyme loading of the polymer of greater than approximately 0.1 percent by weight of the polymer, and including a sufficient amount of surfactant in the reaction mixture to increase enzyme activity at the enzyme loading.
>
> Havens et al disclose immobilizing an enzyme in a polyurethane polymer by synthesizing the polymer in a reaction mixture containing the enzyme and a surfactant. Loading of enzyme of at least 0.1 wt% would have been inherent in method of Havens et al since the polymer may contain 5 mg of protein per gram of prepolymer resin (page 2256, right col, line 17 from the bottom) which is 0.5 wt of protein%, and the crude enzyme extract is partially purified by ammonium sulfate precipitation (page 2256, left col, first complete paragraph). The surfactant used by Havens et al would have inherently provided increased enzyme activity at the enzyme loading.

The Examiner further asserted that the arguments and declaration by Keith E. Lejeune, filed on 11/18/02 by the Applicant, have been fully considered but they are not persuasive.

> Applicants urge that the 0.5 wt% protein in the polymer of Havens et al contains a much lower concentration of enzyme, and based on mathematical calculations presented in the declaration, assert that enzyme loading of the Havens et al polymer is only .0058 wt%.
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> The mathematical calculations are unpersuasive since they are based on the polymer of Havens et al containing 2.9 mg protein per g prepolymer whereas Havens et al disclose that 5 mg protein per g prepolymer may be present. Furthermore, the calculations are based on the rate of reduction in parathion concentration in Figure 1 of Havens et al. However, the rate of reduction in parathion content could have been affected by factors other than only the amount of active enzyme present in the polymer such as the ability of the parathion substrate to contact all active enzyme in the polymer. The polymer could have partially blocked access of the substrate to the entrapped enzyme. Therefore, there could have been substantially more active enzyme in the polymer than appears from the rate of substrate reduction. Furthermore, in addition to the active enzyme, there could have been a substantial amount of inactive enzyme in the polymer. The present claims do not require a certain amount of the loaded enzyme to be active enzyme. The present claims and specification do not require conditions different than used by Havens et al that would have resulted in greater enzyme loading than obtained by Havens et al.

Applicants respectfully traverse the Examiner's rejections.

Once again, Applicants are not claiming merely the addition of surfactant during the synthesis of a polymer immobilizing an enzyme, but including a sufficient amount of a surfactant in the reaction mixture to increase enzyme activity at an enzyme loading than is substantially higher than the enzyme loadings disclosed in Havens et al.

Applicants used the data of 2.9 mg protein per g prepolymer as set forth in Havens et al, because that was the only loading for which data were available. In that regard, in Havens et al., a very crude (impure) protein preparation was used at a

maximum concentration of 5mg/g polymer. Although this is 0.5 wt% of protein preparation, the concentration of enzyme in that preparation is much lower. Although not indicated, the enzyme loading in the polymer of Havens et al. is well less than 0.01 wt%. In that regard, in LeJeune W, earlier cited by the Examiner, a purified enzyme at 0.006mg/g polymer (0.006 wt%) was used. The enzyme activity of the polymer of LeJeune W and Havens et al. is similar, indicating a similar enzyme loading.

Moreover, as set forth in the Declaration of Keith E. LeJeune filed November 12, 2002, the turnover number (or kcat) for the subject enzyme of Havens et al. (Parathion hydrolase from P. diminuta) on parathion is well established as 1,067 umol/min/mg enzyme. (See, for example, Dumas et al., 1989 J. Biol. Chem. 264, 19659-19665 and Dumas et al., 1990 Arch. of Biochem. Biophys., 277, 1, 155-159). Moreover, the inventors of the present application have verified these findings in the laboratory.

In Havens et al, the polymer used to initiate the hydrolysis (shown in Figure 1) is described as a 5.5g polymer carrying 2.9 mg protein / g prepolymer, or approximately 16mg of protein. Havens et al. also describes that the standard assay was to apply substrate solutions to the polymers at 10-times the polymer mass, or in that case 55ml of parathion solution. Figure 1 shows a reduction of parathion concentration from 0.045 to 0.01 nmol/uL in 5 minutes, or a rate of 0.007 nmol/uL/min (or also 7 nmol/mL/min). Multiplying by the volume of 55 ml, one calculates the apparent reaction rate to be 385 nmol/min. Considering 16 mg of the protein prep was required to achieve this rate, the actual observed rate was 24 nmol/min/mg protein.

If one very conservatively assumes a low activity retention of enzyme of 1% during polymerization, the resulting rate in the Havens et al. experiment should have been 10.67 umol/min/mg enzyme, if the protein preparation of Havens et al. was pure enzyme. The division of their achieved rate (24 nmol/min/mg protein) by that expected (10.67 umol/min/mg enzyme) for pure enzyme, provides the purity of the prep (0.024/10.67) or 0.002 mg enzyme/ mg protein. This calculation indicates that the protocols described by Havens et al. employed a protein prep that was less than or equal to 0.2% pure enzyme. Enzyme loading would thus be less than or equal to 0.0058 mg enzyme/ g prepolymer ([0.002 mg enzyme / mg protein] * [2.9 mg protein / g

prepolymer]) or .00058 wt percent enzyme loading (0.0058 mg enyme/1000mg prepolymer * 100). Even assuming an extremely low activity retention of 0.1%, the wt percent enzyme loading of the polymer of Havens et al. would be only .0058 wt%.

Moreover, under the above conservative calculations, even assuming the concentration of impure protein of Havens et al. were in prepolymer were 5.0 mg protein / g prepolymer rather than 2.9 mg protein / g prepolymer, the polymer loading would still be well below the 0.1 percent by weight claimed in the present invention. Moreover, Applicants allowed for the possibility that there is significant inactive enzyme in the materials of Havens et al. and that the physical properties of the polymer may be poor (effectively blocking access to enzyme), by assuming a very conservatively low activity retention of enzyme of 1% during polymerization.

Including a sufficient amount of a surfactant in the reaction mixture to increase enzyme activity at any enzyme loading, let alone the relatively high enzyme loading of the present invention, is not disclosed or suggested in Havens et al. Moreover, contrary to the Examiner's earlier assertion, increased loading of enzyme in a polymer is clearly not "inherent."

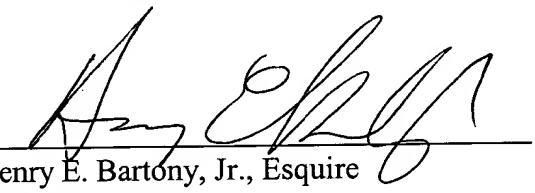
Havens et al. to not disclose or suggest that surfactants, when used in sufficient amounts, increase enzyme activity as claimed in the present invention. Indeed, Applicants are the first to discover that surfactants can be used to increase enzyme activity at the relatively high enzyme loading of the present invention. Havens et al. does not even address the problem of retention of activity at high enzyme loadings. Through increased surfactant in the reaction mixtures of the present invention, relatively large quantities of enzymes are immobilized within the polymers of the present invention while retaining a significant portion of the native enzyme specific activity. Once again, prior to the present invention, high enzyme loadings were not used because sufficient activity was not retained. The present invention thus provides a substantial improvement in the art.

The Examiner further indicated that claims 3 and 4 are allowable, "but are objected to as being dependent on a rejected claim." In light of the above remarks, Applicants respectfully assert that claims 3 and 4 are allowable as written.

In light of the above remarks, Applicants respectfully request that the Examiner withdraw his rejection of Claims 1 and 3-10, and that the Examiner indicate the allowability of Claims 1 and 3-10 and arrange for an official Notice of Allowance to be issued in due course.

Respectfully submitted,

KEITH E. LEJEUNE ET AL.

By 
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